Originals

植物研究雑誌
J. Jpn. Bot.
73: 59–66 (1998)

Karyotype Analysis in the Genus *Asiasarum* F. Maek. (Aristolochiaceae)

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(Received on December 27, 1996)

The somatic chromosome numbers and karyotypes of five species of *Asiasarum* (Aristolochiaceae), representing seven distinct taxa, were studied. The seven taxa had the same somatic chromosome number, 2n=26. With one exception, they had the same chromosome complements, 10m+3st. The exception was *A. maculatum* with 11m+2st. These seven taxa commonly had three pairs of small chromosome, among which one pair of small chromosomes (No. 11) with a satellite, and one pair of small chromosome (No. 12) with a secondary constriction at the distal portion of the short arms. The somatic chromosome number and karyotype of *A. patens*, and *A. sieboldii* var. *versicolor* and karyotype of *A. heterotropoides* var. *mandshuricum* are reported for the first time. The similarity in chromosome number and karyotype suggests that *Asiasarum* is a well-defined phyletic group belonging to *Asarum* sensu lato.

Asiasarum F. Maek. is a herbaceous perennial in the Aristolochiaceae. There are five species, which are found in Korea, Japan and northern China. Maekawa (1936) recognized *Asiasarum* as a distinct genus, based on morphological features including long-stalked stamens, separate non-columnous styles, perfect six-celled ovary, absence of calyx tube, and annual leaves folded in the bud. Several workers however, have been classified it within the genus *Asarum* sensu lato (*Asarum* s. str., *Asiasarum* F. Maek., *Geotaenium* F. Maek., *Heterotropa* Morr. & Decne., and *Hexastylis* Raf.) because of a wide range of variation in morphological characters and the difficulty of generic delimitation (Schmidt 1935, Araki 1937, 1953). These differences in the systematic status of *Asiasarum* may be due to both different taxonomic recognition and the lack of suitable characters to evaluate the relationships between these species (Sugawara 1981).

Comparative studies of *Asarum* s. lat. (including *Asiasarum* species) have made on the basis of morphological (Araki 1937, Sugawara 1987), chemotaxonomical (Fujita 1966), and cytological characters (Tanaka 1935, Gregory 1956, Ono 1960, Maekawa and Ono 1965, Lee 1967, Yuasa and Maekawa 1976, Kim et al. 1978, Sugawara 1981, 1982, 1985a, 1985b, Sugawara and Ogisu 1986, Soltis 1984). Cytological studies have been especially useful in clarifying the relationships among *Asarum* s. lat. (Sugawara, 1981).

Several workers have undertaken cytological studies of the genus (Tanaka 1935, Ono 1960, Lee 1967, Kim et al. 1978, Sugawara 1981). Tanaka determined the somatic chromosome number of *A. dimidiatum* as 2n=24.

Ono (1960) reported that of *Asiasarum sieboldii* as 2n=26 in the study of *Heterotropa* and related genera. Lee (1967) reported the somatic chromosome numbers of *Asiasarum maculatum* [=*Asarum maculatum* (2n=26)] and *A. heterotropoides* var. *mandshuricum* [=*A. sieboldii* subsp. *heterotropoides* (2n=26, 28)]. Kim et al. (1978) also published the somatic chromosome numbers and karyotypes of *Asiasarum maculatum* [=*Asarum maculatum* (2n=26)], *A. sieboldii* var. *sieboldii* [=*A. sieboldii* (2n=26)], *A. heterotropoides* var. *seoulense* [=*A. sieboldii* var. *seoulense* (2n=26)] and discussed the phylogenetic relationship of these species.

Sugawara (1981, 1982, 1987) conducted a taxonomic study of *Asarum* s. lat. on the basis of karyotype characteristics, C-band pattern, and floral anatomy. In a more recent study, Sugawara (1991) included also pollen morphology in considering of phylogenetic relationships among these genera.

The purpose of this study was to determine the interspecific relationships within *Asiasarum* and the intergeneric relationships within *Asarum* s. lat. We examined the somatic chromosome numbers and karyotypes of five species of *Asiasarum*, including a new species and a new variety recognized by Yamaki et al. (1996). We compared our results with previous reports to determine the relationships between *Asiasarum* and closely related genera.

Materials and Methods

Plants used in this study were 130 individuals from 26 populations collected from 24 localities between May 1992 and May 1993. The localities are listed in Table 1. They were identified in reference with Maekawa (1936) and Yamaki et al. (1996). Fresh materials have been planted in the garden of the Department of Biology, Kyungpook National University, Taegu, Korea. Root tips were obtained from these materials and pretreated with 0.002 M 8-hydroxyquinoline solution for four and a half hours. After fixation with 45% acetic acid for 25 minutes, the root tips were macerated in a 1:2 mixture of 45% acetic acid: 1 N hydrocloric acid for 50 sec. at 60°C, and then stained with 1% orcein solution. Subsequently, they were compressed according to the standard method. Karyotypes were arranged according to chromosome size and arm ratio. The arm ratio and the nomenclature for the centromere position on chromosomes were according to the description of Sugawara (1981). Voucher specimens are deposited in the Herbarium of Kyungpook National University (KNU).

Results

The somatic chromosome numbers and karyotypes of seven taxa were determined. The same somatic chromosome number, 2n=26, was found in all taxa: *Asiasarum dimidiatum, A. heterotropoides* var. *mandshuricum, A. heterotropoides* var. *seoulense, A. maculatum, A. patens, A. sieboldii* var. *sieboldii*, and *A. sieboldii* var. *versicolor*. This number agrees with previous reports by Ono (1960), Lee (1967), Kim et al. (1978), and Sugawara (1981) (Table 1). The somatic chromosome numbers and karyotypes of *A. patens* and *A. sieboldii* var. *versicolor* are reported for the first time. The complement of somatic chromosome of *A. heterotropoides* var. *mandshuricum* is also published here for the first time.

The same karyotype composition, 10m+3st, was found in *A. dimidiatum, A. heterotropoides* var. *mandshuricum, A. heterotropoides* var. *seoulense, A. patens, A. sieboldii* var. *sieboldii* and *A. sieboldii* var. *versicolor*. The composition of *A. maculatum* was 11m+2st. The ten pairs of metacentric chromosomes were longer than three pairs of metacentric or subtelocentric chromosomes. The metacentric chromosome pairs Nos. 1 and 2 were longer than the others,

Table 1. Sampling localities and chromosome numbers for species in the genus *Asiasarum* used in this study. Voucher specimens are deposited in the Herbarium of Kyungpook National University (KNU)

Taxa	Localities	Present study (2n)	Previous study (2n)
A. dimidiatum (F. Maek.) F. Maek.	JAPAN. Ehime, Mt. Takanawa (alt. 980m)	26	24 (Tanaka 1935) 26 (Sugawara 1981)
A. heterotropoides (Fr. Schm.) F. Maek. var. *mandshuricum* (Maxim.) F. Maek.	KOREA. Kangwon, Mt. Jewangsan Kyonggi, Kwangrung	26	26 (Lee 1967)
A. heterotropoides var. *seoulense* (Nakai) F. Maek.	KOREA. Kangwon, Ssarijae (alt. 1300m)	26	26 (Kim et al. 1978)
A. maculatum (Nakai) F. Maek.	KOREA. Cheju, Seongpanak, Valley Suakkeigok, Temple Kwaneum-sa, Mt. Hallasan alt. 1100m Cheonnam, Isl. Wando. Kyongnam, Mt. Keumsan	26	26 (Lee 1967, Kim et al. 1978)
A. patens Yamaki	KOREA. Chungnam, Mt. Kyeryongsan Kyongbuk, Mt. Keumosan, Mt. Sanseongsan	26	
A. sieboldii (Miq.) F. Maek. var. *sieboldii*	KOREA. Cheonnam, Mt. Naejangsan, Namchang area. Chungbuk, Mt. Soknisan Kangwon, Valley Murungkeigok Kyongbuk, Mt. Keumosan, Nogojae, Mt. Palgongsan Kyongnam, Mt. Cheonhwangsan, Mt. Chirisan, Mt. Hogusan, Mt. Kayasan	26	26 (Ono 1960, Lee 1967, Kim et al. 1978, Sugawara 1981)
A. sieboldii var. *versicolor* Yamaki	KOREA. Chungbuk, Mt. Soknisan Kangwon, Mt. Chiaksan Kyonggi, Mt. Myongjisan, Mt. Unaksan	26	

and had secondary constrictions on both of their arms. The subtelocentric chromosome, No. 11, had satellites at the distal portion of its short arm. One of the three small chromosome pairs (No. 12) had a secondary constriction at the position of the short arm. The chromosome pair, No. 12, of *A. maculatum* is a metacentric chromosomes. The length of each chromosome for all the *Asiasarum* species examined ranged from 2.75 μm to 13.50 μm.

Asiasarum dimidiatum 2n=26 (Figs. 1A and 2A)

This species is found in Kyushu and Shikoku, Japan. The somatic chromosome number of the plants collected from Mt. Takanawa (alt. 980m), Japan is 2n=26. This agrees with a previous report by Sugawara (1981) (Table 1). Although, Tanaka (1935) reported the somatic chromosome number of *A. dimidiatum* as 2n=24, our study supports that it is 2n=26.

Asiasarum heterotropoides var. *mandshuricum* 2n=26 (Figs. 1B and 2B)

Specimens of this species were collected from Mts. Jewangsan and Kwangrung in Korea. Plants from these populations has a somatic chromosome number of 2n=26. This is the same value as reported by Lee (1967). We

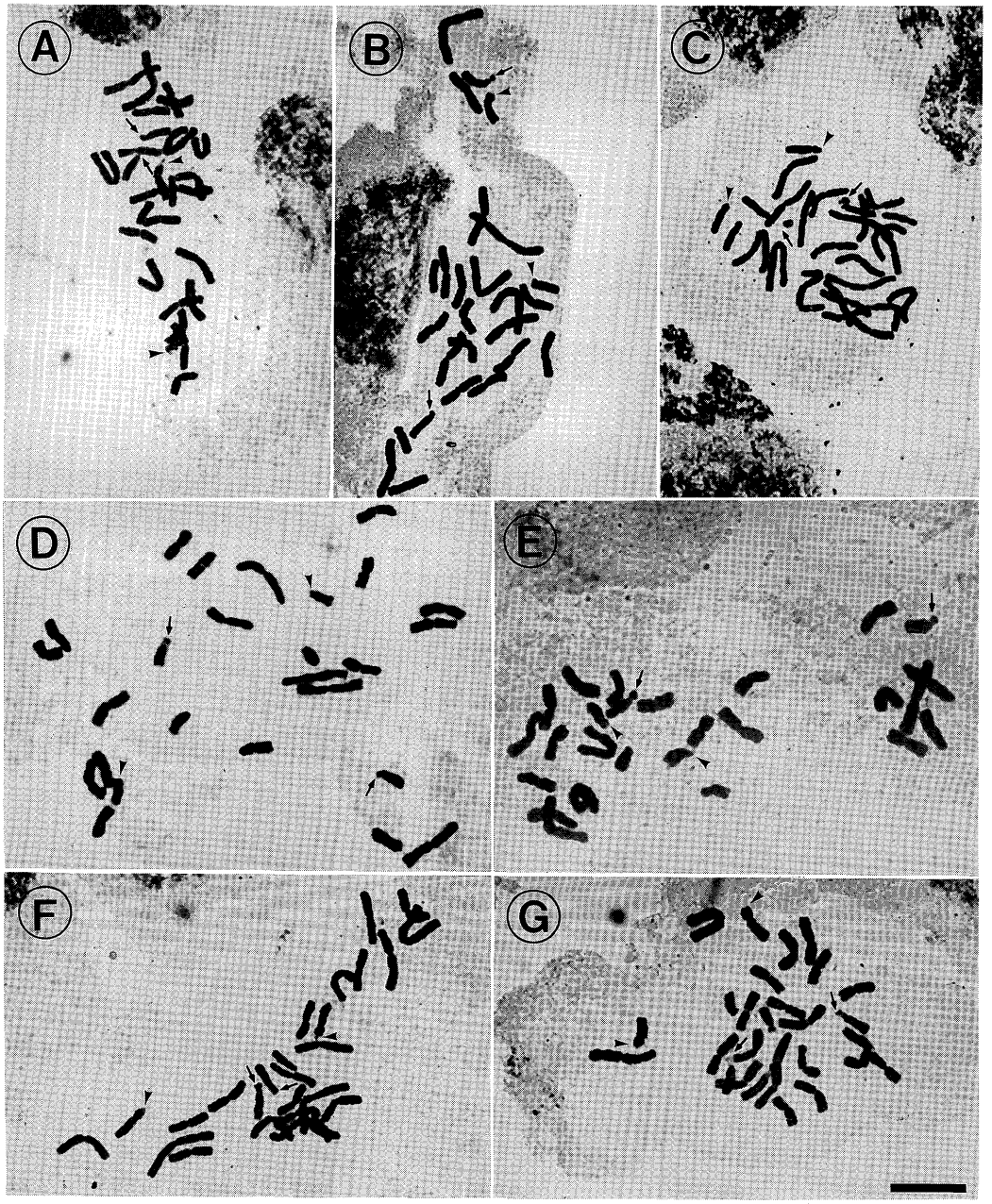

Fig. 1. Photomicrographs of the mitotic metaphase chromosome of *Asiasarum.* Scale bar is 10 μm. Arrows point out the secondary constrictions of small chromosomes. Arrowheads indicate the subtelocentric SAT-chromosomes. A: *A. dimidiatum*, B: *A. heterotropoides* var. *mandshuricum,* C: *A. heterotropoides* var. *seoulense,* D: *A. maculatum,* E: *A. patens,* F: *A. sieboldii* var. *sieboldii,* G: *A. sieboldii* var. *versicolor.*

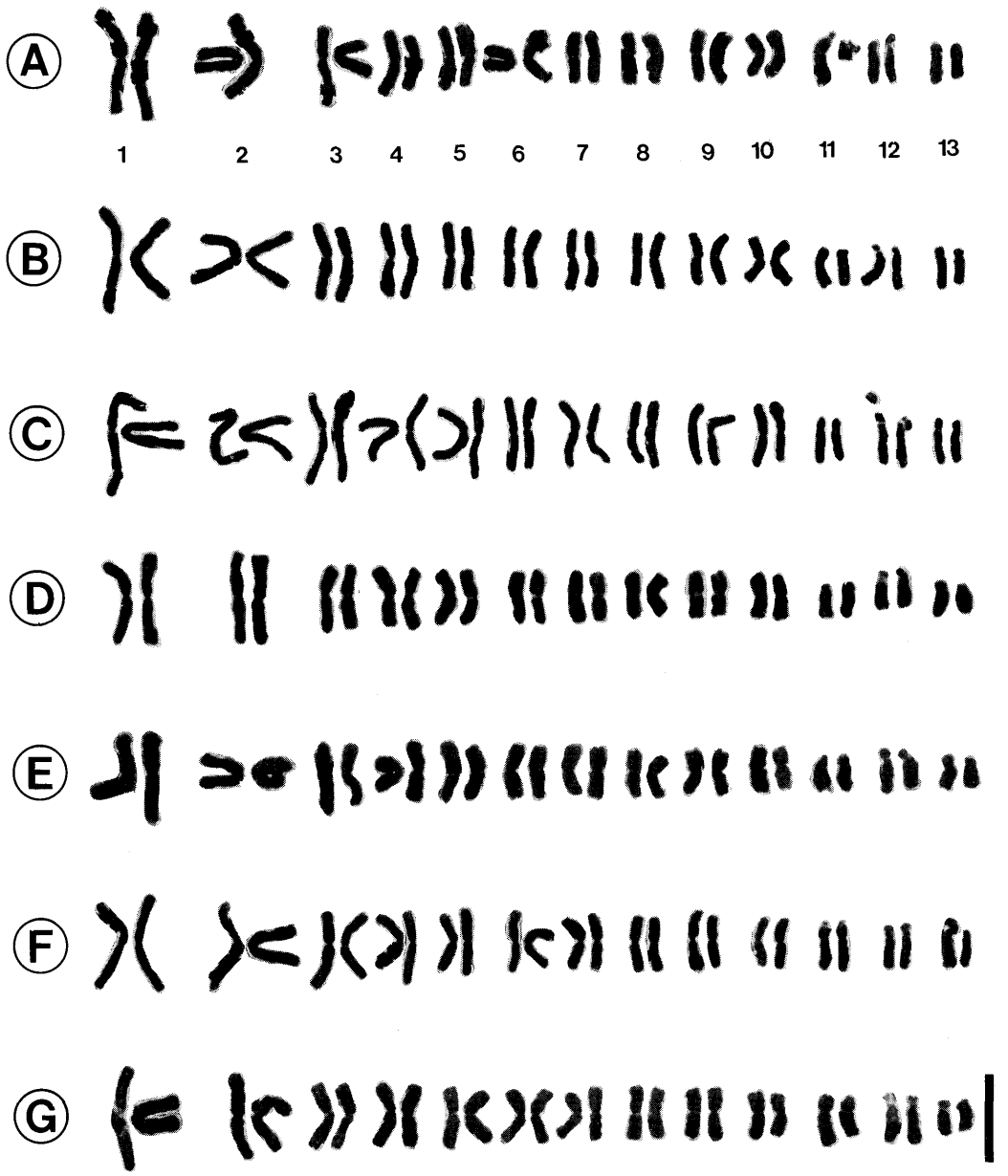

Fig. 2. Karyotypes from the mitotic metaphase of *Asiasarum.* Chromosomes are arranged by size and centromere position. Scale bar is 10 μm. A: *A. dimidiatum,* B: *A. heterotropoides* var. *mandshuricum,* C: *A. heterotropoides* var. *seoulense,* D: *A. maculatum,* E: *A. patens,* F: *A. sieboldii* var. *sieboldii,* G: *A. sieboldii* var. *versicolor.*

report the karyotype for the first time.

Asiasarum heterotropoides var. *seoulense* 2n=26 (Figs. 1C and 2C)

This species, collected from Ssarijae (alt. 1300m) in Korea, has a somatic chromosome number of 2n=26. This number is identical with that reported by Kim et al. (1978) (Table 1). The chromosome complement of *A.*

heterotropoides var. *seoulense* is 10m+3st. This observation differs from that of Kim et al. (1978).

Asiasarum maculatum 2n=26 (Figs. 1D and 2D)

This species occurs in the undergrowth of forests on islands in southern Korea. Specimens were collected from Wando Island, Mt. Keumsan on Namhaedo Island, and Cheju-do Island. All the plants examined have a somatic chromosome number of 2n=26. This agrees with reports by Lee (1967) and Kim et al. (1978) (Table 1). However, we found a different chromosome complement, 11m+2st, from that reported by Kim et al. (1978). They published the karyotype of *A. maculatum* as 2m+9sm+2st.

Asiasarum patens 2n=26 (Figs. 1E and 2E)

This new species, described by Yamaki et al. (1996), was collected from Mt. Keumosan, Mt. Kyeryongsan, and Mt. Sanseongsan in Korea. The somatic chromosome number is 2n=26. This is the first report of the somatic chromosome number and karyotype for this species.

Asiasarum sieboldii var. *sieboldii* 2n=26 (Figs. 1F and 2F)

This species is widely distributed in Korea, Japan and China. Several workers have reported the chromosome number of this species (Table 1). Specimens were collected from Mt. Naejangsan, Mt. Soknisan, the Murungkeigok Valley, Mt. Keumosan, Nogojae, Mt. Palgongsan, Mt. Cheonhwangsan, Mt. Chirisan, Mt. Hogusan, and Mt. Kayasan, Korea. All have a somatic chromosome number of 2n=26 which agrees with those of Ono (1960), Lee (1967), Kim et al. (1978), and Sugawara (1981) (Table 1), although the karyotypes are different. Ono (1960) reported a karyotype of 11m+2st and existence of a satellite. Kim et al. (1978) suggested the chromosome complement as 5m+6sm+2st with the existence of a satellite. Sugawara (1981) reported the chromosome complement as 10m+3st with no satellite.

Asiasarum sieboldii var. *versicolor* 2n=26 (Figs. 1G and 2G)

This species was recognized as a new variety of *A. sieboldii* by Yamaki et al. (1996). Plants from four populations on Mt. Sonkisan, Mt. Chiaksan, Mt. Myongjisan, and Mt. Unaksan, Korea have a somatic chromosome number of 2n=26. The number and karyotype are published for the first time for this taxon.

Discussion

Sugawara (1981) studied the chromosome numbers and karyotypes of *A. dimidiatum, A. heterotropoides*, and *A. sieboldii.* He characterized *Asiasarum* as having three pairs of subtelocentric chromosomes and no satellites. This observation does not agree with previous reports by Ono (1960) and Kim et al. (1978). Ono (1960) reported that *Asiasarum* was characterized by a single pair of subtelocentric chromosomes. Kim et al. (1978) described the karyotype of *A. maculatum* as 2m+9sm+2st, *A. sieboldii* var. *sieboldii* as 5m+6sm+2st, and *A. heterotropoides* var. *seoulense* as 5m+7sm+1st. Ono (1960) and Kim et al. (1978) both reported the existence of a pair of satellites in these species.

In this study, we examined five species, and seven distinct taxonomic groups, of *Asiasarum.* Our karyotypic data differs from previous reports. All the species examined had the same somatic chromosome complement of 10m+3st, except *A. maculatum* (11m+2st). All species had one pair of small chromosomes with a secondary constriction and one pair of small chromosomes with a satellite at the distal portion of the short arms in common. These species are identical in floral characters (shape of the calyx tube, stamen and style, position of the stigma, the presence of ridges in the calyx tube, etc.).

Asiasarum maculatum has a somewhat bril-

liant, slightly thickened leaf with white spots, unlike the leaves of other *Asiasarum* species. The external morphological characters of the leaf are very similar to those of *Heterotropa*. However, the karyotype of this species is similar to that of *Asarum* s. str., 11m+2st or 12m+1st (Sugawara 1981). Kim et al. (1978) proposed that *A. maculatum* is a primitive *Asisarum* because of the white spots and degenerative chloroplasts of the leaf.

The somatic chromosome complement of *Asiasarum* differs from those of all other taxa of *Asarum* s. lat., except *Hexastylis*. *Hexastylis* has the same somatic chromosome number (2n=26) and karyotype (10m+3st) as *Asiasarum*. However, the two genera have a different number of chromocenter and C-banding patterns (Sugawara 1982). Ono (1960) considers *Hexastylis* as being closely allied to *Asarum* s. str. because of the similarity of their karyotypes.

In conclusion, the similarity in the chromosome number and morphology of *Asiasarum* species, along with their similar floral morphology, indicates that they are a well-defined phyletic group among the *Asarum* s. lat.

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崔 昊[a], 山木克之[b], 寺林 進[b], 岡田 稔[b], 朴 宰弘[a]：ウスバサイシン属植物の核型分析

ウスバサイシン属*Asiasarum*（ウマノスズクサ科 Aristolochiaceae）の5種7分類群；クロフネサイシン *A. dimidiatum* (F. Maek.) F. Maek., ケイリンサイシン *A. heterotropoides* (Fr. Schm.) F. Maek. var.

mandshuricum (Maxim.) F. Maek., ウスゲサイシン *A. heterotropoides* var. *seoulense* (Nakai) F. Maek., アツバカンアオイ *A. maculatum* (Nakai) F. Maek., オオバナサイシン*A. patens* Yamaki, ウスバサイシン *A. sieboldii* (Miq.) F. Maek. var. *sieboldii*, フイリウスバサイシン*A. sieboldii* var. *versicolor* Yamaki について，染色体数と核型を調べた．7分類群の染色体数はいずれも2n＝26であった．核型については，アツバカンアオイは11m＋2st で，他のものはすべて10m＋3st であった．7分類群に共通した特徴として，No. 11の染色体には短腕部にサテライトがあり，No. 12の染色体には短腕部に二次狭窄が観察された．オオバナサイシン，フイリウスバサイシン，ケイリンサイシンについては，染色体数，核型の報告は本報が最初である．これらウスバサイシン属植物の染色体数の一致や核型の類似は，広義のカンアオイ属 *Asarum* s. l.の中でウスバサイシン属がよくまとまった系統群であることを示唆している．

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